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Respiratory virus detection in nasopharyngeal aspirate versus bronchoalveolar lavage is dependent on virus type in children with chronic respiratory symptoms



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ABSTRACT

Background: The comparative yield of respiratory virus detection from nasopharyngeal aspirate (NPA) versus bronchoalveolar lavage (BAL) is uncertain. Furthermore, the significance of virus detection and its relationship to lower airway neutrophilic inflammation is poorly studied.

Objectives: To evaluate the sensitivity, specificity and predictive values of NPA for detecting respiratory viruses in BAL; and to determine the relationship between viruses and lower airway neutrophilia in children with non-acute respiratory illness.

Study design: 150 paired NPA and BAL samples were obtained from 75 children aged <18 years undergoing flexible bronchoscopy for investigation of chronic respiratory symptoms. Viral studies were performed using polymerase chain reaction (PCR). Cellularity studies were performed on BALs. Diagnostic parameters of NPA compared to BAL and associations between viruses and lower airway %neutrophils were evaluated.

Results: NPA had a higher yield than BAL for detection of any respiratory virus (52 versus 38, respectively). NPA had a high sensitivity (92%) and low specificity (57%) for detecting HRV in BAL with poor kappa agreement value of 0.398 (95% CI 0.218–0.578, $p < 0.001$). NPA had a fair sensitivity (69%) and good specificity (90.3%) for detecting HAdV on BAL, kappa agreement was 0.561 (95% CI 0.321–0.801, $p < 0.001$). HAdV positivity on NPA, compared to negativity, was independently associated with heightened airway neutrophilia [mean difference (95% CI): 18 (1,35); $p = 0.042$].

Conclusions: NPA has a higher yield for respiratory virus detection than BAL, however its diagnostic accuracy is dependent on viral species. Adenovirus positivity is associated with significantly heightened lower airway neutrophilia in children with chronic respiratory symptoms.

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1. Background

Accurate identification of respiratory pathogens is important in clinical medicine and research. In bacteriology, upper airway sampling can misrepresent the lower airway microbiota [1,2]. In contrast, there is little such comparative data for virology. Nasopharyngeal aspirates (NPA) or nasal washes are regarded as the specimen of choice for detection of upper respiratory tract viruses by polymerase chain reaction (PCR) [3,4]. Upper airway sampling is relatively simple, can be performed at the bedside and is minimally invasive. In contrast, lower airway sampling

Abbreviations: NPA, nasopharyngeal aspirate; BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; RVI, respiratory virus infection; HRV, human rhinovirus; HAdV, human adenovirus; RSV, respiratory syncytial virus; IFAV, human influenza A virus; IFBV, human influenza B virus; HPIV1–3, human para-influenza virus 1–3; hMPV, human metapneumovirus; HBoV, human bocavirus; HCoV – NL63, OC43, 229E, HKU1, human coronaviruses; WUPyV, WU polyomavirus; KIPyV, KI polyomavirus.

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in young children is invasive, requiring a bronchoalveolar lavage (BAL).

Traditionally, BAL has been considered the ‘gold-standard’ for the microbiological diagnosis (viral and bacterial) of lower respiratory tract infection in children. However, as BAL requires specialist input and is often performed under anesthesia, it is reserved for patients with complex or severe disease, e.g., in the setting of intensive care; immune-compromise or in children with chronic or recurrent respiratory tract symptoms. The disparities in ease of collection of NPA versus BAL, raise two important questions. Firstly, what is the comparative yield of NPA compared to BAL for respiratory virus detection in children? Secondly, what is the significance of a positive NPA with respect to lower airway inflammation? Given the lack of studies addressing these important questions [5,6], we evaluated paired NPA and BAL specimens in 75 children.

2. Objectives

To compare the yield and diagnostic parameters of respiratory virus detection on NPA versus BAL using molecular methods, and to evaluate whether virus positivity on NPA correlates with lower airway neutrophilic inflammation and by inference, active viral pulmonary disease.

3. Study design

The Queensland Children’s Health Services Ethics Committee approved the study and written informed consent was obtained from each parent or guardian. Children undergoing flexible bronchoscopy, for any clinical indication, as arranged by their treating physician, were eligible. Samples evaluated in this article were obtained from children recruited to a larger cohort study on chronic cough in children [7]. Caregivers completed a standardized clinical questionnaire on the day of bronchoscopy including current respiratory symptoms and relevant demographics. Children with symptoms of significant acute lower respiratory tract infection, e.g., high fever, tachypnea/shortness of breath, wheeze or rattly chest were deemed, by an anesthetist, to be unfit for anesthesia and excluded from the study.

Contemporaneous NPA and BAL sampling was performed under general anesthesia. NPA was collected first, using a disposable catheter connected to a mucous trap. Dry nasopharyngeal suction, via both nares, was performed, followed by suction of 2–3 ml of sterile normal saline directly into the suction catheter to rinse through remaining contents.

Bronchoscopic BAL was then performed using standardized methods as per European Respiratory Society guidelines [8]. Sterile normal saline, in three aliquots of 1 ml/kg (maximum 20 ml), was instilled into the most affected area, or right middle lobe in patients with generalized disease. To minimize upper airway contamination, suction through the bronchoscope was avoided until the tip had entered the distal airways. The first aliquot was used for microbiological testing, the second and third were pooled for cellularity studies.

Viral studies were undertaken using real-time PCR techniques, as described previously [9–13], to detect 16 respiratory virus types and subtypes. These included human rhinoviruses (HRV), human adenoviruses (HAdV), respiratory syncytial virus (RSV), human influenza A virus (IFAV), human influenza B virus (IFBV), human para-influenza virus 1–3 (HPIV1–3), human metapneumovirus (hMPV), human bocavirus (HBoV), human coronaviruses (HCoV – NL63, OC43, 229E, HKU1), WU polyomavirus (WUPyV) and KI polyomavirus (KIPyV). BAL specimens were refrigerated immediately and processed within 24 h. NPA specimens were stored at –70 °C prior to processing.

We defined positive bacterial culture as growth of $\geq 10^5$ colony-forming units (cfu)/ml [10] of any of the following pathogens on BAL: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* or Enterobacteriaceae. Total cell count and differential profile (minimum 400 cells counted) were performed on BAL as described previously [14].

3.1. Statistical analyses

Statistical analyses were carried out using IBM SPSS (v20, San Francisco, USA). Medians and inter-quartile ranges were reported as data were non-normally distributed. Kappa analyses were used to assess levels of agreement between sampling techniques. Comparisons of categorical variables were performed using Pearson’s χ^2 test or Fisher exact test if any category had an expected value of <5. Mann–Whitney *U* test was used for two group comparisons and Kruskal–Wallis test for >2 groups comparisons of continuous variables. Multiple linear regression was employed to adjust for virus co-detection and bacterial infection. A two-tailed *p*-value <0.05 was considered statistically significant. Sensitivity, specificity, positive and negative predictive values and likelihood ratios were manually calculated.

4. Results

Between February 2010 and May 2012, paired NPA and BAL samples were obtained from 75 children (median age = 33 months, IQR 16, 69; male *n* = 50, 66.7%). One-third of children (*n* = 25, 33.3%) were exposed to household cigarette smoke. Chronic cough (≥ 4 weeks) was reported in 45 participants (60%) at time of bronchoscopy. The major diagnoses within the cohort included: protracted bacterial bronchitis (*n* = 31, 41%) and bronchiectasis (*n* = 17, 23%); the remaining participants had either no specific diagnosis (*n* = 22, 29%) or a congenital airway abnormality (*n* = 5, 7%). The most common viruses detected on NPA were HRV (*n* = 44) and HAdV (*n* = 15). In regard to seasonality, HAdV positivity on BAL was more likely to occur in spring or summer than winter or autumn (*p* = 0.004) using chi-square analysis. HRV detections followed a bimodal distribution with peak detections occurring in spring and autumn.

4.1. Comparison of yield on paired NPA and BAL samples

Of the 75 paired NPA and BAL samples, 52 (69.3%) children had one or more virus detected from NPA and 38 (50.7%) had one or more virus from BAL. When considering detection of any virus, 32 (42.7%) NPA-BAL pairs were concordant PCR-positive, 17 (22.7%) pairs were concordant PCR-negative and 26 (34.7%) pairs had discordant PCR results. Of the discordant pairs, most (*n* = 20, 76.9%) were PCR positive on NPA but negative on BAL. Detection rates varied among the different viruses (Table 1). Most marked was the difference between NPA and BAL for HRV. Using positive PCR on BAL as an arbitrary gold standard, we calculated sensitivity, specificity, positive and negative predictive values and likelihood ratios (Table 1). HRV and HAdV were the most common viruses detected from NPA and BAL. Levels of agreement between NPA and BAL varied among the different viruses. The agreement between NPA and BAL for HRV was poor, kappa = 0.398 (95%CI 0.218, 0.578, *p* < 0.001) but good for HAdV at 0.561 (95%CI 0.321, 0.801, *p* < 0.001).

4.2. Relationship between virus positivity and BAL neutrophilia

The median lower airway %neutrophils were significantly higher in children who were NPA-positive for any virus compared to

Table 1Absolute number of virus detections by PCR from paired NPA/BAL samples ($n = 75$) with diagnostic test parameters (BAL as the arbitrary gold-standard).

NPA/BAL	HRV	HAdV	Other ^a	HBoV	HCoV	WUPyV/KIPyV	Total ^b
NPA+	44	15	5	7	4 ^c	4	52
BAL+	24	13	4	4	3 ^c	1	38
NPA+/BAL–	22	6	1 ^d	5	3	4	20
NPA–/BAL+	2	4	0	2	2	1	6
NPA+/BAL+	22	9	4	2	1	0	32
NPA–/BAL–	29	56	70	66	69	70	17
Sensitivity	92%	69%	–	50%	33%	–	84%
Specificity	57%	90%	99%	93%	96%	–	46%
PPV	0.5	0.6	0.80	0.29	0.25	–	0.62
NPV	0.94	0.93	–	0.97	0.97	–	0.74
LR+	2.12	7.15	–	7.10	7.99	–	1.56
LR–	0.19	0.34	–	0.54	0.70	–	0.35
Kappa	0.40	0.56	0.88	0.32	0.25	0.02	0.30

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR–, negative likelihood ratio.

^a RSV, IFA/B, HPIV1–3, hMPV.^b Total no. of children with one or more viruses detected.^c HCoV-OC43.^d HPIV-1.**Table 2**

Lower airway cellular differential versus virus status on NPA and BAL.

Lower airway cellular profile	NPA HRV+ ($n = 44$)	NPA HRV– ($n = 31$)	p -Value	NPA AdV+ ($n = 15$)	NPA AdV– ($n = 60$)	p -Value
TCCs ($\times 10^6/L$)	215 (106, 285)	240 (94, 360)	0.894	260 (168, 473)	200 (90, 290)	0.132
Neutr%	16 (6, 55)	13 (4, 32)	0.514	51 (15, 68)	12 (5, 36)	0.017
Macr%	60 (37, 82)	71 (29, 87)	0.605	46 (23, 74)	71 (41, 84)	0.075
Lymph%	6 (4, 13)	6 (3, 15)	0.812	5 (2, 11)	6 (4, 15)	0.183
Eosin%	0 (0, 0)	0 (0, 0)	0.262	0 (0, 2)	0 (0, 0)	0.314
Lower airway cellular profile	BAL HRV+ ($n = 24$)	BAL HRV– ($n = 51$)	p -Value	BAL AdV+ ($n = 13$)	BAL AdV– ($n = 62$)	p -Value
TCCs ($\times 10^6/L$)	210 (108, 413)	230 (95, 350)	0.645	240 (112, 485)	215 (94, 285)	0.534
Neutr%	16 (9, 63)	15 (5, 40)	0.457	44 (14, 70)	12 (5, 37)	0.077
Macr%	65 (27, 82)	68 (39, 84)	0.544	54 (25, 83)	70 (38, 84)	0.497
Lymph%	8 (5, 20)	5 (3, 14)	0.318	3 (2, 7)	7 (5, 15)	0.017
Eosin%	0 (0, 2)	0 (0, 0)	0.162	0 (0, 1)	0 (0, 0)	0.825

Abbreviations: Neutr%, percentage neutrophils; Macro%, percentage macrophages; Lymph%, percentage lymphocytes; Eosin%, percentage eosinophils. Cell counts expressed as median (interquartile range) and rounded to nearest whole number.

NPA-negative children (19 [IQR 7, 65] vs. 11 [IQR 3, 21.5]; $p = 0.045$). Total cell counts (TCCs), %macrophages, %lymphocytes and %eosinophils were similar across groups (data not shown). When evaluating the two most frequent viruses, HRV and HAdV (Table 2), presence of HAdV in the NPA was significantly associated with increased BAL neutrophilia ($p = 0.017$) whereas HRV status on NPA showed no association with %neutrophils ($p = 0.514$).

Similar to the NPA findings, the presence of any virus in the BAL was associated with higher median %neutrophils compared to the virus-negative state (23 [IQR 10, 67] vs. 11 [5, 28], respectively; $p = 0.04$). Median lower airway TCC was higher in BAL virus-positive ($235 \times 10^6/L$ [IQR 115, 448]) versus virus-negative ($160 \times 10^6/L$ [58, 255]) groups ($p = 0.068$). No differences in lymphocyte counts was observed when considering total viruses, but for individual viruses, %lymphocytes were significantly lower in HAdV BAL positive compared to HAdV BAL negative participants ($p = 0.017$). Children who were HAdV positive on BAL had higher %neutrophils than those who were HAdV negative on BAL ($p = 0.077$). In comparison, %neutrophils were similar across HRV positive and negative groups on BAL ($p = 0.457$).

4.3. Regression analysis adjusting for virus co-detection and bacterial infection

On multi-variable analysis, HRV in either NPA or BAL showed no association with lower airway %neutrophils. In contrast, HAdV in NPA and/or BAL was significantly associated with increased %neutrophils on BAL (Table 3). Bacterial infection was detected in over

half (32; 61.5%) of NPA virus positive participants with much lower rates (6; 26.1%) in virus negative participants ($p = 0.005$). Rates of bacterial infection in BAL virus positive (22; 57.9%) children were also greater than BAL virus negative children (16; 43.2%); however, this difference was not significant ($p = 0.204$). The predominant bacterial organisms were: *H. influenzae*, *M. catarrhalis* and *S. pneumoniae*. Presence of bacterial infection as seen on BAL had the strongest independent influence on lower airway neutrophil counts (beta = 0.256 and 0.311 for NPA and BAL, respectively) as shown in Table 3.

5. Discussion

In this study, we systematically compared upper airway (NPA) to lower airways (BAL) sampling with regards to diagnostic yield for respiratory viruses in children with non-acute respiratory illness. We found that concordance of NPA with BAL is largely dependent on the virus being investigated. NPA has good concordance with BAL for detection of HAdV, but poor concordance for HRV. In comparison to BAL, sensitivity, specificity, PPV and NPV of the most frequently detected viruses on NPA differed according to the virus being investigated. Specificity was high for all viruses, except for HRV. The reverse was observed with regards to sensitivity. Lastly, presence of HAdV on NPA and/or BAL was associated with significantly heightened %neutrophils in the lower airway.

To our knowledge, three major studies have compared upper and lower airway specimens for respiratory virus detection. All were limited by unpaired specimens or focused on specific

Table 3
Multiple linear regression of mean difference in lower airway %neutrophils according to infective status on NPA and BAL.

	Unadjusted ^a mean difference in neutr% (IQR)	p-Value	Adjusted ^b mean difference in neutr% (IQR)	p-Value ^c	Beta ^d
NPA					
HAdV	20 (3, 37)	0.020	18 (1, 35)	0.042	0.245
HRV	5 (–9, 19)	0.486	–2 (–16, 12)	0.782	–0.033
Bacteria $\geq 10^5$ cfu/ml (on BAL)	16 (3, 30)	0.016	15 (1, 28)	0.035	0.256
BAL					
HAdV	15 (–3, 32)	0.097	18 (0.3, 35)	0.046	0.235
HRV	4 (–10, 19)	0.550	0 (–14, 15)	0.950	0.007
Bacteria $\geq 10^5$ cfu/ml (on BAL)	16 (3, 30)	0.016	18 (4, 31)	0.01	0.311

^a Unadjusted mean difference in %neutrophils between HAdV, HRV and bacteria positive and negative groups calculated using independent samples *t*-test.

^b Adjusted mean difference in %neutrophils using multiple linear regression (adjusted for viral co-detection and bacterial infection).

^c *p*-Values remain significant after logarithmic transformation of %neutrophils therefore mean differences reported.

^d Standardized coefficient indicates independent influence of variable on %neutrophils.

diseases or viruses. The first compared NPA to BAL in lung transplant recipients using unpaired, non-contemporaneous NPA and BAL specimens obtained from 72 and 56 participants for NPA and BAL, respectively. A higher yield of hMPV was seen in NPA compared to BAL, with comparable rates of IFAV, HPIV, RSV, HCoV and HRV [6]. Diagnostic accuracy of NPA for BAL was not assessed in this study [6]. The second study used paired specimens, obtained from 21 participants on 92 occasions, and compared induced sputum to NPA for detection of respiratory viruses in children with cystic fibrosis. HRV was the most common virus identified and was present in the same frequency (21.7%) in NPA and sputum. Concordance rates were higher than in the present study (87% for HRV and 92% for other viruses), with higher sensitivity and specificity (70% and 91.7%, respectively) [15]. The third study compared paired nasal wash/tracheal suction specimens to BAL in terms of RSV detection in 6 participants, and found BAL was superior for detection of RSV using antigen detection and culture methods (insensitive methods when compared to PCR) [5].

Studies directly comparing NPA to BAL have focused on atypical pneumonia. The first study evaluated *Pneumocystis jiroveci* pneumonia and the second compared detection methods for mycoplasma pneumonia. Both studies found high concordance between NPA and BAL. However, in contrast to our findings, in both studies, BAL had a higher yield of positive detections than NPA. Interestingly, in the study on mycoplasma pneumonia, BAL positivity was associated with increased lower airway %neutrophils, however, NPA positivity was not. These differing findings may reflect the different modes of transmission and mechanisms of disease progression between viral and bacterial infections of the respiratory tract.

Our study showed that NPA/BAL discordance was most marked for HRV, where 22 (29.3%) children were NPA-positive but BAL-negative. In contrast, HAdV was more likely to also be found in the lower airway, with only 2 NPA-positive/BAL-negative children. Previous studies have found HRV to have greater propensity to infect the nasopharynx and proximal lower airways than the distal airways or alveoli [16]. This may be related to HRV's ability to replicate optimally at cooler temperatures of the upper airway [17], although this finding is inconsistent across HRV types [18]. In vivo studies demonstrate that HRV induces minimal epithelial cytotoxicity with only a small subset of nasal and bronchial mucosal cells becoming infected [16,19,20]. These factors may explain why HRV is an uncommon cause of pneumonia, except in the immune-compromised host [21]. Whilst HRV and HAdV can both cause uncomplicated upper respiratory tract infection [22], disease manifestation differs at the severe end of the spectrum. HRV has been shown to play an important role in asthma exacerbations [23]; HAdV has been implicated in the pathogenesis of obliterative bronchiolitis and bronchiectasis [24]. Thus, differences

between HAdV and HRV in their mechanisms of propagation and respiratory disease pathogenesis are likely to explain our observations.

Our cross-sectional findings of elevated rates of HRV (59%) in children who were essentially free of acute RVI symptoms is higher than prior studies showing asymptomatic HRV nasopharyngeal detection ranging from 12% to 33% [25–27]. HRV is frequently identified in patients with viral:bacterial co-infection [28], a fact that may have relevance to our cohort of children with high rates of bacterial infection and chronic cough.

To further understand the implications of our findings in relation to lower airway neutrophilic inflammation and virus detection, the distinction between positive virus detection and active viral disease merits discussion. A PCR-positive NPA in a child without overt symptoms of acute RVI is often believed to represent viral genome shedding from a resolving infection [29] or preceding a new symptomatic episode. However, certain HAdV types can cause prolonged host shedding [30] with intermittent viral excretion over months to years [31,32]. In contrast, HRVs usually do not persist. Garnett et al. postulate that “smoldering HAdV at the site of lung inflammation” may contribute to the pathogenesis of lung diseases such as chronic obstructive pulmonary disease (COPD) in adults [33]. Our finding of low BAL %lymphocytes, together with neutrophilia, in children who were HAdV DNA positive on BAL, supports the notion that active lower airway HAdV replication was likely to be occurring (as opposed to latent HAdV infection of lymphocytes). Whether such persistence of HAdV viral DNA within the respiratory tract of children is benign or contributes to the development of chronic lung disease/s is unknown.

The major strengths of this study are its prospective design, the use of highly sensitive PCR techniques to detect a wide range of respiratory viruses, contemporaneous detection of lower airway bacteria and inclusion of BAL cellularity findings. However, there are a number of limitations. Firstly, this study involved children with chronic respiratory symptoms, predominantly cough. Hence, these findings may not be readily extrapolated to healthy children or to those with acute lower respiratory tract illness. This is particularly pertinent to influenza and RSV, as there were very few children in whom these viruses were detected. Secondly, we used neutrophilic lower airway inflammation as a surrogate marker for active pulmonary disease, as used by other authors [34,35]. We did not, however, test for additional indirect markers of viral infection such as Toll-like receptor 3 (TLR3), interferon gamma induced protein 10 (IP-10) or virus-specific antibodies. Thirdly, we only obtained BAL samples at a single time-point, precluding the ability to make inferences regarding causality. Lastly, although we were fastidious in our BAL technique, contamination of the bronchoscope in its route through the upper airway is possible. The significant number of BAL positive/NPA negative cases, however, negates the

argument that contamination could account for all lower airway detections.

In conclusion, we have shown that while upper airway sampling is the most practical and frequently used method of assessing virus presence, it does not necessarily reflect virus in the lower airways in children with non-acute respiratory illness. However, certain viruses (e.g., HAdV), when detected on NPA, may provide clues to the existence of lower airway neutrophilic inflammation and further research is needed. Studies addressing acute viral respiratory tract illnesses in children are required to ascertain the applicability of our findings to the acute setting.

Author contributions

Dr. Wurzel co-conceptualized the study, was responsible for data analysis and manuscript preparation and was involved in data collection.

Dr. Marchant co-conceptualized the study and assisted in data analysis and manuscript preparation.

Dr. Clark provided intellectual input into the study and critically reviewed the manuscript.

Dr. Mackay and Ms. Wang were responsible for viral processing of specimens and critically reviewed the manuscript.

Prof. Sloots was responsible for viral processing of specimens and critically reviewed the manuscript.

Prof. Upham and Dr. Yerkovich assisted in preparation and critical review of the manuscript.

Assoc. Prof. Masters assisted in data collection and critical review of the manuscript.

Dr. Baker assisted in statistical aspects of the study.

Ms. Anderson-James assisted with data collection and study coordination.

Prof. Chang conceptualized the study and was involved in all aspects of the study.

All authors read and approved the final manuscript as submitted.

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Competing interests

IM has received payment for consultancy services from Firebrick pharma. JU has served on the advisory board for Novartis and has previously received payments for lectures or served on speakers' bureaus for AstraZeneca, GSK, Novartis and Boehringer.

All other authors report no potential conflicts.

Ethical approval

Ethical approval was given by Queensland Children's Health Services (RCH) Human Research Ethics Committee (HREC). Reference number: HREC/03/QRCH/17.

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References

- [1] Goddard AF, Staudinger BJ, Dowd SE, Joshi-Datar A, Wolcott RD, Aitken ML, et al. Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of upper-airway specimens can misrepresent lung microbiota. *Proceedings of the National Academy of Sciences of the United States of America* 2012;109:13769–74.
- [2] Armstrong DS, Grimwood K, Carlin JB, Carzino R, Olinsky A, Phelan PD. Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. *Pediatric Pulmonology* 1996;21:267–75.
- [3] Hyypia T, Puhakka T, Ruuskanen O, Makela M, Arola A, Arstila P. Molecular diagnosis of human rhinovirus infections: comparison with virus isolation. *Journal of Clinical Microbiology* 1998;36:2081–3.
- [4] Ngaosuwanikul N, Noisumdaeng P, Komolsiri P, Pooruk P, Choekphaibulkit K, Chotpitayasonondh T, et al. Influenza A viral loads in respiratory samples collected from patients infected with pandemic H1N1, seasonal H1N1 and H3N2 viruses. *Virology Journal* 2010;7:75.
- [5] Englund JA, Piedra PA, Jewell A, Patel K, Baxter BB, Whimbey E. Rapid diagnosis of respiratory syncytial virus infections in immunocompromised adults. *Journal of Clinical Microbiology* 1996;34:1649–53.
- [6] Gerna G, Vitulo P, Rovida F, Lilleri D, Pellegrini C, Oggionni T, et al. Impact of human metapneumovirus and human cytomegalovirus versus other respiratory viruses on the lower respiratory tract infections of lung transplant recipients. *Journal of Medical Virology* 2006;78:408–16.
- [7] Chang AB, Yerkovich ST, Gibson PG, Anderson-James S, Petsky HL, Carroll ML, et al. Pulmonary innate immunity in children with protracted bacterial bronchitis. *Journal of Pediatrics* 2012;161:621–5, e1.
- [8] de Blic J, Midulla F, Barbato A, Clement A, Dab I, Eber E, et al. Bronchoalveolar lavage in children. ERS Task Force on bronchoalveolar lavage in children. *European Respiratory Society, European Respiratory Journal* 2000;15:217–31.
- [9] Syrmis MW, Whitley DM, Thomas M, Mackay IM, Williamson J, Siebert DJ, et al. A sensitive, specific, and cost-effective multiplex reverse transcriptase-PCR assay for the detection of seven common respiratory viruses in respiratory samples. *Journal of Molecular Diagnostics* 2004;6:125–31.
- [10] Bialasiewicz S, Whitley DM, Lambert SB, Gould A, Nissen MD, Sloots TP. Development and evaluation of real-time PCR assays for the detection of the newly identified KI and WU polyomaviruses. *Journal of Clinical Virology* 2007;40:9–14.
- [11] Tozer SJ, Lambert SB, Whitley DM, Bialasiewicz S, Lyon MJ, Nissen MD, et al. Detection of human bocavirus in respiratory, fecal, and blood samples by real-time PCR. *Journal of Medical Virology* 2009;81:488–93.
- [12] Mackay IM, Arden KE, Speicher DJ, O'Neil NT, McErlan PK, Greer RM, et al. Co-circulation of four human coronaviruses (HCoV) in Queensland children with acute respiratory tract illnesses in 2004. *Viruses* 2012;4:637–53.
- [13] Mackay IM. Human rhinoviruses. In: Schuller M, Sloots TP, James GS, Halliday CL, Carter IWJ, editors. *PCR for clinical microbiology: an Australian and international perspective*. London: Springer; 2010.
- [14] Marchant JM, Gibson PG, Grissell TV, Timmins NL, Masters IB, Chang AB. Prospective assessment of protracted bacterial bronchitis: airway inflammation and innate immune activation. *Pediatric Pulmonology* 2008;43:1092–9.
- [15] Burns JL, Emerson J, Kuypers J, Campbell AP, Gibson RL, McNamara S, et al. Respiratory viruses in children with cystic fibrosis: viral detection and clinical findings. *Influenza and Other Respiratory Viruses* 2012;6:218–23.
- [16] Mosser AG, Vrtis R, Burchell L, Lee WM, Dick CR, Weisshaar E, et al. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *American Journal of Respiratory and Critical Care Medicine* 2005;171:645–51.
- [17] Killington RA, Stott EJ, Lee D. The effect of temperature on the synthesis of rhinovirus type 2 RNA. *Journal of General Virology* 1977;36:403–11.
- [18] Papadopoulos NG, Sanderson G, Hunter J, Johnston SL. Rhinoviruses replicate effectively at lower airway temperatures. *Journal of Medical Virology* 1999;58:100–4.
- [19] Arruda E, Boyle TR, Winther B, Pevear DC, Gwaltney Jr JM, Hayden FG. Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. *Journal of Infectious Diseases* 1995;171:1329–33.
- [20] Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, et al. Rhinoviruses infect the lower airways. *Journal of Infectious Diseases* 2000;181:1875–84.
- [21] Ison MG, Hayden FG, Kaiser L, Corey L, Boeckh M. Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. *Clinical Infectious Diseases* 2003;36:1139–43.
- [22] Denny Jr FW. The clinical impact of human respiratory virus infections. *American Journal of Respiratory and Critical Care Medicine* 1995;152:S4–12.
- [23] Johnston SL, Pattermore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *British Medical Journal* 1995;310:1225–9.
- [24] Becroft DM. Bronchiolitis obliterans, bronchiectasis, and other sequelae of adenovirus type 21 infection in young children. *Journal of Clinical Pathology* 1971;24:72–82.
- [25] Jansen RR, Wieringa J, Koekkoek SM, Visser CE, Pajkrt D, Molenkamp R, et al. Frequent detection of respiratory viruses without symptoms: toward

- defining clinically relevant cutoff values. *Journal of Clinical Microbiology* 2011;49:2631–6.
- [26] Advani S, Sengupta A, Forman M, Valsamakis A, Milstone AM. Detecting respiratory viruses in asymptomatic children. *Pediatric Infectious Disease Journal* 2012;31:1221–6.
- [27] van der Zalm MM, Wilbrink B, van Ewijk BE, Overduin P, Wolfs TF, van der Ent CK. Highly frequent infections with human rhinovirus in healthy young children: a longitudinal cohort study. *Journal of Clinical Virology* 2011;52:317–20.
- [28] Franz A, Adams O, Willems R, Bonzel L, Neuhausen N, Schweizer-Krantz S, et al. Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection. *Journal of Clinical Virology* 2010;48:239–45.
- [29] Pavia AT. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. *Clinical Infectious Diseases* 2011;52:S284–9.
- [30] Brandt CD, Kim HW, Vargosko AJ, Jeffries BC, Arrobio JO, Rindge B, et al. Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. *American Journal of Epidemiology* 1969;90:484–500.
- [31] Fox JP, Brandt CD, Wassermann FE, Hall CE, Spigland I, Kogon A, et al. The virus watch program: a continuing surveillance of viral infections in metropolitan New York families. VI. Observations of adenovirus infections: virus excretion patterns, antibody response, efficiency of surveillance, patterns of infections, and relation to illness. *American Journal of Epidemiology* 1969;89:25–50.
- [32] Fox JP, Hall CE, Cooney MK. The Seattle Virus Watch. VII. Observations of adenovirus infections. *American Journal of Epidemiology* 1977;105:362–86.
- [33] Garnett CT, Erdman D, Xu W, Gooding LR. Prevalence and quantitation of species C adenovirus DNA in human mucosal lymphocytes. *Journal of Virology* 2002;76:10608–16.
- [34] Jarjour NN, Gern JE, Kelly EA, Swenson CA, Dick CR, Busse WW. The effect of an experimental rhinovirus 16 infection on bronchial lavage neutrophils. *Journal of Allergy and Clinical Immunology* 2000;105:1169–77.
- [35] Grigg J, Riedler J, Robertson CF. Bronchoalveolar lavage fluid cellularity and soluble intercellular adhesion molecule-1 in children with colds. *Pediatric Pulmonology* 1999;28:109–16.